

POSTER PRESENTATION

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Integrating neighbor clustering, coexpression clustering and subsystems analysis to define dynamic changes in regulatory networks associated with group A streptococcal sociomicrobiology and niche adaptation

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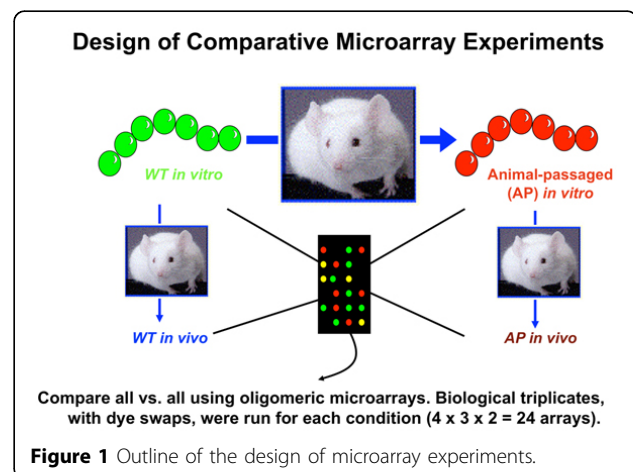
Background

Bacterial colonies often consist of heterogeneous communities rather than genetically identical cells with harmonized gene expression profiles[1]. Dramatic changes, such as the onset of infection, may perturb a colony's sociomicrobiology leading a minor subpopulation with a mutant phenotype to prevail in the host; however, capturing such transitions in real time is difficult. While differential microarray analysis has become a method of choice for comparing the transcriptomes of bacterial subpopulations, current microarray analysis tools are more optimized to the study of eukaryotic organisms.

Here, we set out to develop a systems biology model for studying the transcriptional reprogramming underlying the transition of M1T1 group A streptococci[2] from a virulent to a hypervirulent phenotype [3-5]. In addition, we aimed at integrating and optimizing microarray analysis strategies to better understand bacterial regulatory networks.

Materials and methods

Using a murine subcutaneous chamber model developed in our laboratory[6], we sampled the bacteria before and 24 h after infection, and we compared the transcriptomes of wild type (WT) and animal-passaged (AP) bacteria *in vitro* and *in vivo* (Figure 1). To make biologically relevant discoveries without compromising the statistical



robustness of microarray analysis, we combined multiple analysis strategies, including coexpression clustering, neighbor clustering[7], and subsystems analysis[8]. Finally, we visually inspected all computer-generated data to filter out any artifacts or errors.

Results and conclusion

The integration of these methods revealed extensive transcriptional alterations of pathoadaptive and metabolic gene sets associated with invasion, immune evasion, and metabolic reprogramming. Comparing genomic subsystems involved in *in vivo* adaptation with those affected by animal-passage demonstrates that the WT bacteria struggled to adapt to the host environment

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by altering several virulence and metabolic modules that made their phenotype more similar to the mutant AP phenotype; still, WT bacteria failed to survive while the AP population thrived mainly because of a mutation in the gene encoding their global environmental sensor, CovS. We suggest that the transcriptional reprogramming of the WT bacteria was not sufficient for their adaptation to the host environment and that niche adaptation was only achieved by the selection/expansion of *covS* mutants.

This model allowed us to capture transcriptional snapshots of both survivor and extinct subpopulations *in vivo* and provided a proof of principle that *in vivo* transcriptomics of extinct microbial populations offers valuable clues to microbial niche adaptation. The integration of multiple microarray analysis tools and the customization of these tools to bacterial polycistronic transcripts demonstrate the importance taking biological relevance into consideration in high-throughput data analyses.

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A detailed study describing the experimental model and the implementation of these methods in the analysis of group A streptococcal hypervirulence was recently published[9].

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References

1. Parsek MR, Greenberg EP: **Sociomicrobiology: the connections between quorum sensing and biofilms.** *Trends Microbiol* 2005, **13**:27-33.
2. Aziz RK, Kotb M: **Rise and persistence of global M1T1 clone of *Streptococcus pyogenes*.** *Emerg Infect Dis* 2008, **14**:1511-1517.
3. Aziz RK, Pabst MJ, Jeng A, Kansal R, Low DE, Nizet V, Kotb M: **Invasive M1T1 group A *Streptococcus* undergoes a phase-shift in vivo to prevent proteolytic degradation of multiple virulence factors by SpeB.** *Mol Microbiol* 2004, **51**:123-134.
4. Sumbly P, Whitney AR, Graviss EA, DeLeo FR, Musser JM: **Genome-wide analysis of group A streptococci reveals a mutation that modulates global phenotype and disease specificity.** *PLoS Pathog* 2006, **2**:e5.
5. Walker MJ, Hollands A, Sanderson-Smith ML, Cole JN, Kirk JK, Henningham A, McArthur JD, Dinkla K, Aziz RK, Kansal RG, et al: **DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection.** *Nat Med* 2007, **13**:981-985.
6. Kazmi SU, Kansal R, Aziz RK, Hooshdaran M, Norrby-Teglund A, Low DE, Halim AB, Kotb M: **Reciprocal, temporal expression of SpeA and SpeB by invasive M1T1 group A streptococcal isolates in vivo.** *Infect Immun* 2001, **69**:4988-4995.

7. Ryan PA, Kirk BW, Euler CW, Schuch R, Fischetti VA: **Novel algorithms reveal streptococcal transcriptomes and clues about undefined genes.** *PLoS Comput Biol* 2007, **3**:e132.
8. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, Cohoon M, de Crecy-Lagard V, Diaz N, Disz T, Edwards R, et al: **The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes.** *Nucleic Acids Res* 2005, **33**:5691-5702.
9. Aziz RK, Kansal R, Aronow BJ, Taylor WL, Rowe SL, Kubal M, Chhatwal GS, Walker MJ, Kotb M: **Microevolution of group A streptococci in vivo: capturing regulatory networks engaged in sociomicrobiology, niche adaptation, and hypervirulence.** *PLoS ONE* 2010, **5**:e9798.

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